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FULL-LENGTH ORIGINAL RESEARCH

Epilepsia®

Guideline-based and bioinformatic reassessment of lesion-associated gene and variant pathogenicity in focal human epilepsies

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Summary

Objective: Increasing availability of surgically resected brain tissue from patients with focal epilepsy and focal cortical dysplasia or low-grade glioneuronal tumors has fostered large-scale genetic examination. However, assessment of pathogenicity of germ line and somatic variants remains difficult. Here, we present a state-of-the-art evaluation of reported genes and variants associated with epileptic brain lesions.

Methods: We critically reevaluated the pathogenicity for all neuropathology-associated variants reported to date in the PubMed and ClinVar databases, including 101 neuropathology-associated missense variants encompassing 11 disease-related genes. We assessed gene variant tolerance and classified all identified missense variants according to guidelines from the American College of Medical Genetics and Genomics (ACMG). We further extended the bioinformatic variant prediction by introducing a novel gene-specific deleteriousness ranking for prediction scores.

Results: Application of ACMG guidelines and in silico gene variant tolerance analysis classified only seven of 11 genes to be likely disease-associated according to the reported disease mechanism, whereas 61 (60.4%) of 101 variants of those genes were classified as of uncertain significance, 37 (36.6%) as being likely pathogenic, and 3 (3%) as being pathogenic.

Significance: We concluded that the majority of neuropathology-associated variants reported to date do not have enough evidence to be classified as pathogenic. Interpretation of lesion-associated variants remains challenging, and application of current ACMG guidelines is recommended for interpretation and prediction.

KEYWORDS

focal cortical dysplasia, focal epilepsies, gene pathogenicity, low-grade epilepsy-associated tumors, variant pathogenicity

1 | INTRODUCTION

Somatic gene variants have been increasingly detected and reported in brain tissue obtained from patients with epilepsy-associated focal lesions and considered causal for the lesion and the patient's epilepsy. A correct interpretation of pathogenicity is essential to unravel the genetic variety of epilepsy-associated syndromes and serves as a basis to develop precision treatment. The most common structural brain lesions comprise focal cortical dysplasia (FCD) and low-grade epilepsy-associated tumors,[1–3] both of which represent umbrella terms for a variety of diagnostically related but histologically independent etiologies. FCD is a heterogeneous group of cortical malformations accounting for the most common structural brain lesions within the broad spectrum of malformations of cortical development.[4–9] FCD is diagnosed in up to 18% of patients who undergo epilepsy surgery,[3] mostly affects the frontal lobe, and can histopathologically present with a large spectrum of abnormalities, including cortical architecture, bizarre neuronal cell morphology, blurred gray-white matter boundaries, and heterotopic neurons or increased oligodendroglial cell densities in white matter.[10] The most frequent tumors in patients with drug-resistant focal epilepsy starting in the first 2 decades of life are gangliogliomas and dysembryoplastic neuroepithelial tumors, accounting for 69% of 2244 tumors collected at the European Epilepsy Brain Bank.[3] Intriguingly, these tumors histopathologically present with a variable mixture of glial and neuronal cell lineages and have mostly affected the temporal lobe.[11]

Unlike recent large-scale studies on rare and common germ line variant-associated epilepsies,[12] genetic studies on focal brain lesions comprise so far only reasonably powered hypothesis-free exome-wide gene discovery screens using small cohorts of patients. Patient ascertainment is challenging, because the disease-associated variants are expected to be present only in the brain or even in a fraction of the lesional brain tissue.[2] In addition to the limited access to the target tissue, the prevalence of somatic mosaicism in the human brain of healthy individuals is not well understood, and thus, current small cohort studies without large control sets might lead to biased or even false conclusions. Even for genuine disease-associated genes, not all observed variants would contribute to disease etiology. Due to time and cost constraints, functional testing can usually be conducted only for a minor part of variants observed in patients. The latter represents a more general problem that holds true for all epilepsies, because the accurate interpretation of variation in disease genes has largely lagged behind the massive upscaling of data generation enabled by the increased accessibility of sequencing. However, lesional epilepsies represent a group where variant interpretation even in established disease-associated genes is challenging due to the lack of somatic variant reference databases and lack of variant interpretation guidelines as well as the potential interplay of somatic and germ line variants.

Key Points

- Interpretation of germ line and somatic variants obtained from patients with focal epilepsy and histopathologically confirmed lesions remains challenging
- In silico gene variant tolerance analysis classified only seven of 11 genes to be likely neuropathology-associated according to the reported disease mechanism
- ACMG guidelines and a novel developed deleteriousness ranking classified only 39.6% of neuropathology-associated variants as pathogenic or likely pathogenic
- Interpretation of disease-associated variants was improved by the application of current ACMG guidelines including bioinformatic pathogenicity prediction
- Variants of uncertain significance remain the largest group of variants, and novel high-throughput methods for functional testing are needed

Many recent genetic studies in, for example, familial hypercholesterolemia[13] and maturity onset diabetes of the young[14] have shown that previous disease-associated variants are not pathogenic applying current American College of Medical Genetics and Genomics (ACMG) guidelines. The cost to patients when a classification is incorrect can be a false and missed diagnosis, probably leading to misdirected treatment.

To address the challenging interpretation of neuropathology-associated genes and missense variants, both germ line and somatic, we systematically reevaluated the pathogenicity of genes and variants that have previously been reported to be associated with histopathologically confirmed epileptic brain lesions. We used recent guidelines published by the ACMG including a novel bioinformatic variant assessment approach. Because somatic mutations are present only in a small fraction of brain cells, to cause severe neuropathologies, we expect that their predicted functional effect should be at least as severe as observed for disease-causing germ line variants. The variant evaluation for both germ line and somatic variants was based on ACMG guidelines. One part of the ACMG evaluation represents in silico variant functional prediction. To improve variant evaluation, we developed a novel bioinformatic

approach for in silico variant assessment. The guideline-based analysis represents state-of-the-art assessment of all reported neuropathology-associated variants and supports the verification and identification of disease-associated risk genes and variants in epilepsies. Furthermore, the evaluation procedure can be reapplied to other variant sets not exclusively restricted to epileptic neuropathologies.

2 | MATERIALS AND METHODS

2.1 | Gene and variant identification

Although the evaluation of pathogenicity for loss-of-function (LoF) variants (eg, full gene deletions or nonsense variants) is straightforward, the criteria to establish pathogenicity for missense variants rely on supportive genetic data and functional evidence.[15] We focused, therefore, on the guideline-based[15] interpretation of heterozygous dominant acting neuropathology-associated missense variants and performed a PubMed-based literature review (https://www.ncbi.nlm.nih.gov/pubmed; accessed February 2017) to identify studies reporting genes and dominantly acting neuropathology-associated missense variants. First, we used single search terms as well as two- or three-word combinations of the following keywords: "focal cortical dysplasia," "ganglioglioma," "dysembryoplastic neuroepithelial tumor," "neuropathology," "genetics," "somatic," and "mutations." Second, we collected all missense variants in genes reported to be associated with these neuropathologies in the ClinVar database (http://www.ncbi.nlm.nih.gov/clinvar; release: January 8, 2017) using filters described in Appendix S1. We removed copy number, synonymous, frameshift, splice site, and nonsense variants from the dataset.

2.2 | Assessment of gene variant tolerance

We evaluated literature-reported neuropathology-associated genes for variant tolerance using the *pLI* and missense *z* conservation scores (http://exac.broadinstitute.org/) and compared the results with the proposed pathomechanisms in the recent literature. These scores use a depletion of variants in a gene when compared to the expectation under neutral evolution. The expectation has been estimated from a population reference cohort of >60 000 individuals (http://exac.broadinstitute.org/) as an indication of purifying selection, rendering variants affecting these genes more likely to be implicated in disease etiology.[16] Following the authors' recommendations, we considered genes with *pLI* scores > 0.9 as being intolerant for LoF mutations and those with *z* scores > 3.09 intolerant for missense mutations. We used the missense *z* conservation score when disease variants were reported to be missense variants in the brain lesion, *pLI* when disease variants were reported to be LoF variants, and both when missense and LoF variants were reported to play a role in the disease etiology. Additionally, as an alternative assessment of a gene's impact on diseases, we compared the neuropathology-associated gene set with cancer driver genes from Martincorena et al,[17] who studied the landscape of positive and negative selection in somatic evolution in cancer and systematically cataloged cancer genes.

2.3 | Variant classification

All identified neuropathology-associated somatic and germ line missense variants were classified in accordance with 28 criteria defined by guidelines from the ACMG.[15] These ACMG guidelines were developed to set standards for the interpretation of sequence variants primarily as an educational resource for clinical laboratory geneticists to help them provide quality clinical laboratory services. The ACMG recommends the use of specific standard terminology—"pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign"—to describe variants identified in genes that cause Mendelian disorders. The applied ACMG guidelines represent a collection of 28 criteria and recommend that variant classification must be dependent upon scientific evidence and weighted according to the type of evidence available. ACMG criteria take functional studies, segregation studies, comparison of the variant frequency in patients versus the general population, clinical correlation between gene and clinical features of the patient, inferences based on knowledge of the gene or protein structure, in silico predictions, and other pieces of evidence into account to evaluate variant pathogenicity.

We used the online tool provided by Kleinberger et al[18] to aid the missense variant interpretation process and implemented our gene-specific deleteriousness ranking in the ACMG guidelines by modifying the criteria "PP3 = Multiple lines of computational evidence support a deleterious effect on the gene or gene product." We used the algorithm incorporated in the tool to assign either pathogenicity or a nondeleterious impact based on the selected evidence categories, resulting in three positive variant groups in our study: (1) variants of uncertain significance (VUS), (2) likely pathogenic, and (3) pathogenic variants.

2.4 | Gene-specific deleteriousness ranking based on prediction scores

Current bioinformatic variant prediction scores are not gene-specific and do not inform whether the patient variant score is exceptional compared to scores observed for variants from unaffected individuals in the same gene. To

evaluate gene-specific deleteriousness, we calculated gene-specific score ranks (%) of neuropathology-associated missense variants. We sorted and ranked each patient variant score along scores for singleton germ line missense variants in the same gene identified in the general population from the Genome Aggregation Database (gnomAD v2.0; http://gnomad.broadinstitute.org; February 2017). We calculated deleteriousness ranks (%) of neuropathology-associated missense variants for the three most commonly used variant prediction algorithms, namely CADD,[19] PolyPhen-2_HVAR,[20] and GERP[21] from dbNSFP (dbNSFP v3.3; February 2017).[22] The three scores are commonly used bioinformatic prediction tools, whereof CADD and PolyPhen-2 incorporate multiple different biological and evolutionary scores and GERP exclusively scores conservation across species. Variants that were ranked in the top 10% of the specific gene in at least two of the three prediction tools were considered to have multiple lines of bioinformatic evidence in the ACMG pathogenicity classification.

Barplots and stacked barplots were generated with R software, version 3.3.1.[23]

3 | RESULTS

3.1 | Bioinformatic assessment of gene variant tolerance

In our literature and ClinVar review, we identified a total of 11 genes associated with heterozygous dominant acting neuropathology-associated missense and LoF variants, namely *AKT3*,[24,25] *BRAF*,[1] *DEPDC5*,[26–28] *FGFR1*,[29] *MTOR*,[2,24,30,31] *NPRL2*,[28,32] *NPRL3*,[28,32] *PIK3CA*,[24,25] *PTEN*,[33] *TSC1*,[34] and *TSC2*,[27] in 12 studies. In six of 12 studies (50%), the authors sequenced the whole exome; in three studies (25%), gene panels of 3-14 genes were used, and in another three studies (25%), the authors sequenced only a single gene (Table S1). Only one-half of 12 whole exome sequencing and targeted sequencing studies (50%) list detailed variant calling parameters, and one-half list all variants passing their filter criteria.

Based on the literature, we categorized *TSC1* and *PTEN* as intolerant for LoF, *MTOR*, *PIK3CA*, *BRAF*, and *AKT3* as intolerant for missense, and *NPRL2*, *NPRL3*, *TSC2*, *FGFR1,* and *DEPDC5* as intolerant for both (Table 1). Gene conservation scores (*pLI* and missense *z)* and gene positive selection classification in cancer genomes[17] indicated that only seven (*AKT3, BRAF, DEPDC5, MTOR, PIK3CA, PTEN*, and *TSC1*) of 11 genes showed support for association with a severe disease in early childhood for the reported disease mechanism by harboring significant less[16] or more[17] variants than expected under neutral evolution.

Somatic and germ line disease-associated missense variants have been reported in 10 of 11 neuropathology-implicated genes (Tables S2 and S4). For the remaining gene, *DEPDC5*, only somatic and germ line LoF variants have been associated with neuropathologies so far.[26,35] We next classified exclusively missense variants according to recently published ACMG guidelines including our novel developed gene-specific deleteriousness ranking. The classification of LoF variants in a gene depleted for LoF variants like *DEPDC5* is straightforward because of the expected pathomechanism "haploinsufficiency." Our literature and database review identified 101 neuropathology-associated missense variants in total (Figure 1A; Table S4). Of these, 40 (located in the *AKT3*, *BRAF*, *FGFR1*, *MTOR, PIK3CA,* and *PTEN* genes; 39.6%) were classified as "likely pathogenic" or "pathogenic" by meeting a combination of the following ACMG criteria: showed a damaging effect on the gene or gene product in functional studies, were located in a mutational hot spot and/or critical and well-established functional domain, were absent from controls, had received a missense z score $\geq$ 3.09, showed a deleterious effect in multiple lines of computational evidence, and/or were reported as pathogenic in a reputable source (Table S4). The VUS group comprised 61 missense variants present in all 10 neuropathology-associated genes, representing the majority (60.4%) of identified neuropathology-associated missense variants. Considering somatic and

TABLE 1 Gene variant tolerance

Gene	LoF intolerance in controls	Functional support	Under positive selection in cancer
LoF intolerance			
PTEN	✓ (0.98)	Yes	Glioblastoma
TSC1	✓ (1)	Yes	—
Missense intolerance			
AKT3	✓ (3.95)	Yes	—
BRAF	✓ (3.99)	Yes	Thyroid
MTOR	✓ (7.89)	Yes	Kidney
PIK3CA	✓ (5.42)	Yes	Glioblastoma
LoF/missense intolerance			
DEPDC5[a]	✓/✓ (1/3.29)	Yes	—
FGFR1	✓/– (0.99/2.8)	Yes	—
TSC2	✓/– (1/0.89)	Yes	Liver
NPRL2	–/– (0.35/1.86)	No	—
NPRL3	–/– (0.47/0.37)	No	—

Depletion score analysis of 11 neuropathology-associated genes according to the reported pathomechanism in the literature (either LoF or missense).
✓, intolerant for reported mutations ($pLI \geq 0.9$; $z \geq 3.0$); –, tolerant for reported mutation ($pLI < 0.9$, $z < 3.09$); LoF, loss of function; No, reported pathomechanism without functional support; Yes, reported pathomechanism with functional support, positively selected gene (driver) in cancer genome.[17]
[a]Genes were excluded from the following evaluation of variant pathogenicity, because LoF variants were exclusively found for the phenotypes of interests.

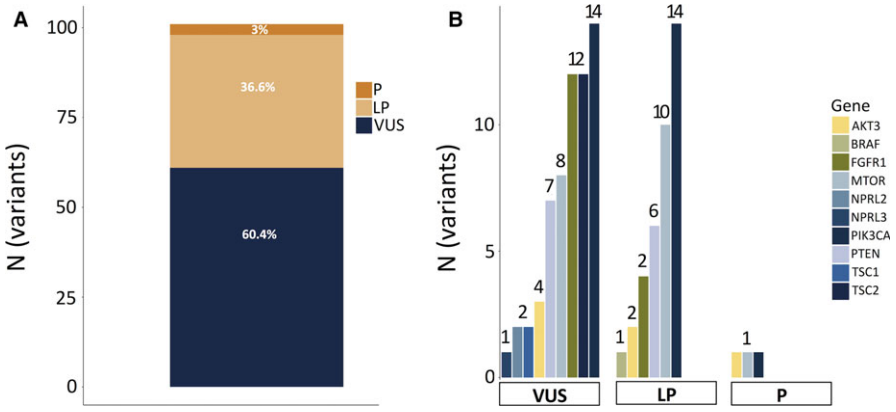

FIGURE 1 American College of Medical Genetics and Genomics criteria classify neuropathology-associated variants as being of uncertain significance (VUS), as being likely pathogenic (LP), or as being pathogenic (P) variants. A, Number of neuropathology-associated LP, P, and VUS variants. B, Amounts of neuropathology-associated LP, P, and VUS missense variants in 10 neuropathology-associated genes. For *DEPDC5,* only loss-of-function variants were reported to be neuropathology-associated

germ line variants separately led to virtually identical variant distributions (Table S2).

3.2 | Gene-specific deleteriousness ranking based on prediction scores

We developed a novel gene-specific deleteriousness ranking approach to improve the in silico missense variant prediction in ACMG classification. The ranking, being based on CADD, PolyPhen-2, and GERP scores, identified 61.2% (60/98) of variants as having higher pathogenic scores than 90% of gnomAD reference variants in at least one score, of which 35% (21/60) had higher pathogenic scores than 90% of gnomAD reference variants in two scores and 10% (6/60) in three scores (Figure 2). Of all neuropathology-associated missense variants, 26.7% (27/101) showed higher pathogenic scores than 90% of gnomAD reference variants in the specific gene in at least two scores for which we applied the ACMG criterion, "Multiple lines of computational evidence support a deleterious effect on the gene or gene product." Three variants do not have gene-specific deleteriousness ranks, as they are not annotated as nonsynonymous variants in dbNSFP.

4 | DISCUSSION

The majority of epileptic brain lesion-associated variants and genes have been identified and classified before variant interpretation guidelines were common practice. Correct classification of variants as pathogenic or benign has a direct benefit to patients. Aside from patient management, certainty in the variant classification will reduce the emotional stress for patients. The objective of this study was to

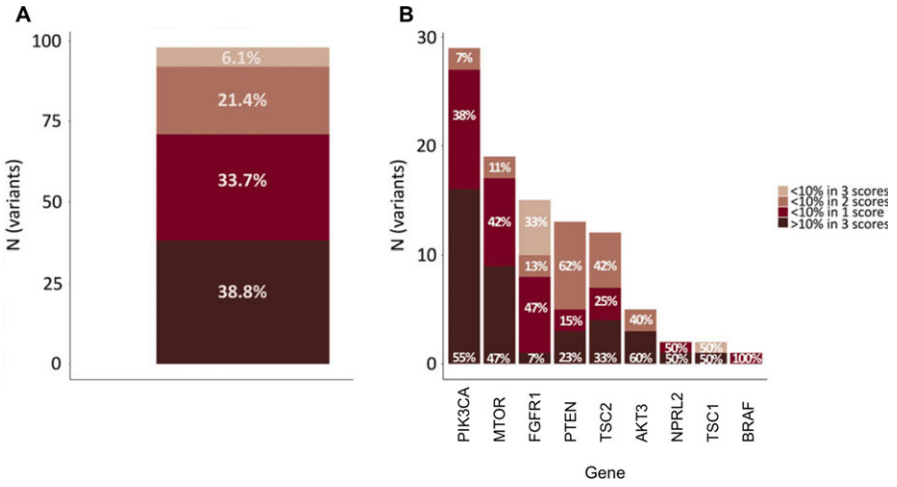

FIGURE 2 Gene-specific deleteriousness ranking of neuropathology-associated variants. A, Summary of variants with CADD, GERP, and PolyPhen-2 scores ranked less pathogenic than 10% of gnomAD controls (>10% in three scores), ranked within the 10th percentile of gnomAD variants in one of the three prediction scores (<10% in one score), ranked within the 10th percentile of gnomAD variants in two of the three prediction scores (<10% in two scores), and ranked within the 10th percentile of gnomAD variants in all three prediction scores (<10% in three scores). B, CADD, GERP, and PolyPhen-2 rank score summary of variants in 9 neuropathology-associated genes (*NPRL3* variants were not annotated in dbNSFP; for *DEPDC5,* only loss-of-function variants were reported to be neuropathology-associated)

reevaluate pathogenicity classification of all described variants and genes associated with epileptic brain lesions according to current guidelines and to establish the proportion of variants that lack evidence to support their pathogenicity. We performed a systematic literature review to identify genes and missense variants identified in patients with epilepsy-associated focal brain lesions. We used ACMG guidelines, including a novel developed bioinformatic method, for the reevaluation of genes and missense variant pathogenicity. Based on our evaluation, we confirmed pathogenicity for only seven of 11 previously reported genes according to the reported pathomechanism and 40 (39.6%) of 101 reported variants.

Classification as disease genes is so far sufficiently supported for seven of the 11 tested genes when using a gene variant tolerance assessment; *AKT3*, *BRAF*, *DEPDC5*, *MTOR*, *PTEN*, *PIK3CA*, and *TSC1* have been identified as disease genes based on sufficient genetic and molecular evidence. All seven genes are missense and/or LoF intolerant and have been linked to the hyperactivation of the mTOR signaling pathway by functional tests (Tables 1 and S3). More proposed disease genes and variants associated with neuropathologies have been identified using targeted sequencing of only mTOR pathway genes with insufficient evidence (no enrichment in exome, no in vivo or in vitro support; Tables S1 and S3). Accordingly, recent studies reporting new disease-associated genes and variants were a priori hypothesis-based and potentially biased. For example, dominantly acting LoF variants in *NPRL2* and *NPRL3* have been reported in neuropathology patients[28] and, correspondingly, haploinsufficiency as the pathomechanism has been proposed. A hallmark of haploinsufficient genes is the absence of LoF variants in healthy individuals.[16] However, *NPRL2*- and *NPRL3*-affecting LoF variants have been reported in unaffected individuals and, for both genes, no statistically significant depletion of variants has been observed in large-scale databases of healthy individuals from the general population. In contrast, at least three papers reported germ line missense, splicing, and LoF variants in *NPRL3* in patients with FCDs, but only targeted sequencing was performed. Proof for the association of these genes with the disease will require further statistical enrichment and in vivo modeling.

We did not find reliable evidence for 61 missense variants to be classified as likely pathogenic by applying ACMG classification criteria including our gene-specific deleteriousness ranking.

Although our results challenge the conclusions from the authors in the individual studies, our observation is not unexpected. Large-scale sequencing has entered the health care sector, and an exponential growth of identified variants observed in databases has begun. Only a small fraction of variants are functionally tested for pathogenicity. It is not surprising that 41.81% of all variants in the patient variant database ClinVar are classified as VUS. Nevertheless, classification of variants as VUS does not rule out that the variant might be pathogenic or benign and that future reevaluation procedures (eg, population studies, segregation studies) or functional tests will reveal or reject variant pathogenicity.

Tremendous advances in sequencing technologies foster variant discovery at an accelerating pace, whereas clinical classification of variants remains in its infancy. This is particularly true for somatic variants in brain diseases, because reference databases for somatic variation in healthy individuals are lacking. Furthermore, the consequences of somatic mosaicism critically result from the diversity, admixture, and developmental stage of neuroepithelial cell types in a given brain tissue across humans. Variants, being pathogenic when present in the germ line, could be benign when present only in a small fraction of cells, when present in a specific cell type where the gene is not expressed, and/or when present with a specific expression at a specific developmental stage. Therefore, we conclude that it will be important to include additional criteria such as the information about gene expression in different tissues in the ACMG guidelines in the future to improve variant evaluation and that it will be especially essential to create reference databases for somatic variation in healthy individuals with the help of single-cell DNA sequencing.

Based on our presented data, pathogenicity interpretation for novel missense variants is feasible only for the minority of variants at this point. Our study further illuminates the uncertainty of prediction of pathogenicity in the absence of sufficient evidence defined by ACMG guidelines. ACMG criteria including statistics or a relatively large number of phenotypically similar patients carrying a mutation in the same gene can give confidence of pathogenicity. In summary, consensus standards for variant assessment, together with results from large-scale research projects, such as the human cell atlas[36] and high-throughput mutagenesis screening, will improve variant interpretation in the near future and importantly will help to improve clinical decision making.

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DISCLOSURE OF CONFLICTS OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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